Transfer Functions of the Output Promoters of Inducible Systems

Overnight Incubation

Complete for each strain in a biohood. Throughout the procedure it is important to label all materials so that the strains do not get confused.

1. Pipette 4 mL sterile LB/antibiotic media to a 14 mL culture tube for each strain. Also pipette 4 mL sterile LB media to a 14 mL culture tube for DH10B.
    a. Yale cells – 4mL LB + 4 µL kan + 4 µL CM
    b. Delta aceE control – 4mL LB + kan
    c. Constructs in DH10B – 4mL LB + 4 µL CM
    d. DH10B – 4mL LB
2. Using a sterile tip transfer a single colony from frozen stock to the corresponding culture tube.
3. Place the cap on the culture tubes to the first position to allow aeration.
4. Place the tubes into the incubator and allow them to grow overnight (~ 16 hours) at 37°C and 250 rpm.

Dilution

5. Measure OD at 600 nm in cuvettes for each culture ($OD_{O/N}$) (900 µL water and 100 µL culture well mixed)
6. Dilute the cultures to OD of 0.1 in a total of 4 mL fresh LB/antibiotic media (just LB for the DH10B) in new 14mL culture tubes.
    a. Yale cells use kan + CM
    b. Delta aceE use kan
    c. Constructs in DH10B use CM
    d. DH10B uses no anitbiotic

    Volume of overnight culture ($V_{O/N}$) = 0.1*4mL/$ODo_{/N}$

    Volume of LB/ antibiotic (just LB for the DH10B) = 4mL – $V_{O/N}$
7. Incubate 1.5 - 2 hours at 37°C and 250 rpm.
8. Measure OD at 600 nm in cuvettes for each culture ($OD_{DIL)}$ and record.

Induction (can do steps 9 - 15 while waiting for step 7)

9. For each inducer, label 3 50mL conicals with the 125x serial dilutions of the maximum inducer concentration, Table 1.
    a. Each set of cells (Yale cells or DH10B cells) will require a separate set of conical

		Tube 9	~~Tube 8~~	~~Tube 7~~	**Tube 6**	~~Tube 5~~	~~Tube 4~~	**Tube 3**	~~Tube 2~~
Inducer (I)	Stock [I]	**Max [I]**	~~5x [I]~~	~~25x [I]~~	**125x [I]**	~~625x [I]~~	~~3125x [I]~~	**15625x [I]**	~~78125x [I]~~
aTc	10^5 ng/mL	250 ng/mL	~~50 ng/mL~~	~~10 ng/mL~~	2 ng/mL	~~400 pg/mL~~	~~80 pg/mL~~	16 pg/mL	~~3.2 pg/mL~~

Table 1 Diluted Inducer Concentrations

aTc

10. Calculate volume to take from the inducer stock to achieve the max [I] for each inducer with the following equation.

    V_{stock} = (**42** mL total in tube) * (250 ng/mL [aTc]) * (4mL total in culture growth tubes)/((10^5 ng/mL [aTc]) *(3.33 mL inducer/LB/ antibiotic mix in tubes)

= 0.1261mL Ara

Inducer	aTc	aTc (using new equation above)
V_{stock}	3 µL	126.1 µL 10^5 ng/mL aTc

Table 2 Inducer Stock and LB/ antibiotic Volume Per Tube 9

11. Add V_{stock} and 42 mL LB/antibiotic to tube 4. Vortex to mix well.
12. Add 41.64 mL LB/antibiotic to tubes 2 and 3.
13. Remove 0.34 mL from each tube 4 and add to tube 3, then vortex. Repeat, 0.2 mL from tube 3 to tube 2, vortexing each time.
14. In 48 tubes, add 3.33 mL from each tube to its corresponding well, see Table 3. In 3 tubes add 3.33 mL LB/antibiotic with no inducer.

pBAD/Ara

Position	Tube 1	Tube 2	Tube 3	Tube 4
Tube and [I]	LB/antibiotic 0 inducer	16 pg/mL atC	2 ng/mL atC	Max [aTc]

Table 3 Inducer Concentration Per Tube

15. Add 3.33 mL LB to Tubes E1, E2 and E3
    a. Add 3.33 mL LB/kan if doing plate 1 (Yale cell control)
16. Add 0.67mL cell culture to the tubes, see Table 3. Add 0.67 mL DH10B to Tubes E1 – E3.
    a. Add 0.67 mL Yale cell control to tubes E4-6 if doing plate 1
17. Put tubes on shaker
18. Incubate 7-8 hours at 37°C and 250 rpm.
19. Measure OD600 of cell cultures in a 96 well plate
20. Spin Cells down at 3200g for 15 minutes, remove supernatant, and freeze at -80°C

	fldA delta aceE1	2	3	fldA-pfo delta aceE1	2	3	petF delta aceE1	2	3	petF-pfo delta aceE1	2	3
	1	2	3	4	5	6	7	8	9	10	11	12
A	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer

B	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2
C	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3
D	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4
E	DH10B 1	2	3	Delta aceE control 1 – no inducer	2 – no inducer	3 – no inducer						
F												
G												
H												

PLATE 1

	fldA 1	2	3	fldA-pfo 1	2	3	petF 1	2	3	petF-pfo 1	2	3
	1	2	3	4	5	6	7	8	9	10	11	12
A	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer	No inducer
B	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2	Tube 2
C	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3	Tube 3

D	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4	Tube 4
E	DH10B 1	2	3									
F												
G												
H												

PLATE 2

Lysis and measurement

21. Suspend frozen pellets in 1 mL of PBS and transfer to 1.5 mL microcentrifuge tubes
22. Sonicate each sample for 60 seconds at 40% amplitude
23. Measure Absorbance at all visible wavelengths in a 96 well plate

Biotin Assay

24. Quant Tag solution: 9.2 mL Reagent 1, 9.2 mL Reagent 2, 920 µL Reagent 3 (should be pale orange color upon mixing all 3) → stable for 8 hours upon mixing
    a. Need 600 µL for standards and 100 µL for each sample (min. 9 mL each)
    b. This is for a duplicate of standards and triplicate of samples
    c. Will make 19.32 mL total of Quant Tag solution
25. Add 5 µL of each sample to a well in a clear 96 well plate
26. Add 0.1 mL of Quant Tag solution to each well and incubate for 30 min at room temperature
27. Measure absorbance of test samples and biotin standards at 535 nm
    a. Blank with Quant Tag only since it is the largest component by far.
    b. Your cells without your plasmids are your “biotin” blank.